Microcapsules

Field of the invention

The present invention relates to microcapsules, and more particularly to microcapsules where an encapsulated aqueous bead or encapsulated aqueous beads comprising the active ingredient or active ingredients is/are further encapsulated in a hydrophobic shell matrix. The present invention relates also to novel methods for preparing the microcapsules according to the invention, as well as to the use of the microcapsules of the present invention.

Background of the invention

US 5,204,029 discloses a process for preparing edible microcapsules which contain a multiplicity of liquid cores. In the process, a water-in-oil emulsion, with the active ingredient dissolved in an inner aqueous phase, is spray cooled, which causes the solidification of the fat phase and the entrapment of the aqueous phase as minute droplets dispersed in a microcapsule. This process, however, leads to very unstable microcapsules from which the water phase migrates from the inner part of the microcapsule to an outer part. This further results in the condensation of the water on the wall of a container.

Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. Vol. 15, pp. 473 to 474, discloses a process in which liquids are encapsulated using a rotating extrusion head containing concentric nozzles. The process is only suitable for liquids or slurries, and the products of the process are large beads having meltable coatings, such as fats or waxes. However, the microcapsules containing a single liquid droplet as a core are very susceptible to rupture.

In their article "Mass preparation and characterisation of alginate microspheres" in Process Biochemistry 35 (2000) 885 to 888 Mofidi, N. et al. describe a method for mass preparation of microspheres, in which method a sterilised alginate solution is prepared and the solution is then poured into a reactor containing a non-aqueous phase, while being stirred. An emulsion of alginate microdroplets is formed and an appropriate amount of the cross-linker is added. Microspheric alginate-gel particles fell to the bottom and they were collected by filtration.

Similarly, Wong, T.W. et al in J. Microencapsulation, 2002 Vol. 19, no 4, 511 to 522, describe release characteristics from pectin microspheres and the method for preparing these microspheres. In this method, pectin microspheres are prepared by a water-in-oil emulsion technique, in which minute droplets of pectin containing an active ingredient dispersed in a liquid hydrophobic continuous phase are hardened and collected by filtration.

Microencapsulation by a coacervation-phase separation process is known from an article by Joseph A. Bakan in Controlled Release Technologies, 1980 by Agis F. Kydonieus. The process consists of a series of three steps carried out under continuous agitation: (1) formation of three immiscible chemical phases; (2) deposition of the coating; and (3) rigidization of the coating.

Sanghvi, S.P. and Nairn J.G. have studied the effect of viscosity and interfacial tension on the particle size of cellulose acetate trimellitate microspheres. The results are presented in their article in J. Microencapsulation, 1992, Vol. 9, no 2, 215 to 227.

In their article in Lebensm. -Wiss. u. -Technol., 33, 80 to 88 (2000) Lee, S.J. and Rosenberg, M. describe a double emulsification and heat gelation process for preparing whey protein-based microcapsules. The microcapsules prepared according to the described process are whey protein-based microcapsules containing an apolar core material.

In their article in Science Vol. 298, 1 November 2002, Dinsmore et al. describe selectively permeable capsules composed of colloidal particles. The capsules are fabricated by the self-assembly of colloidal particles onto the interface of emulsion droplets. After the particles are locked together to form elastic shells, the emulsion droplets are transferred to a fresh continuous-phase fluid that is the same as that inside the droplets.

A disadvantage of the microcapsules or spheres prepared according to the cited references of Lee et al, Dinsmore et al, Mofidi et al or Wong et al is that the microcapsules are only single encapsulated microcapsules and the hydrophobic phase is discarded after the microcapsules have been prepared.

A problem associated with the prior art microcapsules containing only one single liquid phase droplet is that they are very susceptible to rupture. The shell material can break for example during storing or handling of the microcapsules, and this causes the liquid of the whole inner phase to run free. This results in a sticky mass, and the microcapsules are no longer in the form of a free flowing powder.

This problem of rupturing can be somewhat alleviated by preparing microcapsules which contain a multiplicity of liquid cores, as described in the US 5,204,029. However, this process still results in very unstable microcapsules from which the water phase migrates from the inner part of the microcapsule to the outer part and further outside the capsule. This further results in the condensation of water on the wall of the container. Another problem associated with the microcapsules according to the cited US 5,204,029 is that the release of the active ingredient cannot be controlled in the microcapsules.

The present invention seeks to overcome the problems of the known microcapsules, as described above, by providing microcapsules which are very stable and which provide a controlled and/or sustained release of the active ingredient.

Brief description of the invention

The present invention provides microcapsules comprising a solidified hydrophobic shell matrix, encapsulated aqueous bead or beads which is/are further encapsulated in or by the solidified hydrophobic shell matrix, and an active ingredient or active ingredients dissolved or incorporated in the encapsulated aqueous bead or beads, and methods for the preparation thereof, so as to solve the above mentioned problems.

Accordingly, in one aspect, the present invention provides a microcapsule which comprises a solidified hydrophobic shell matrix, an encapsulated aqueous bead or beads encapsulated in or by the solidified hydrophobic shell matrix, and an active ingredient or active ingredients dissolved or incorporated in the encapsulated aqueous bead or beads.

In another aspect, the invention provides a method for preparing microcapsules, which method comprises the steps of

a) providing an aqueous phase and an active ingredient or active ingredients dissolved or incorporated in the aqueous phase,

b) providing a hydrophobic phase in melted form,

c) incorporating or dissolving an encapsulating material or mixture of encapsulating materials in the aqueous phase or in the hydrophobic phase,

d) combining the aqueous phase with the hydrophobic phase and homogenizing or mixing the combined phases to form a water-in-oil emulsion,

e) encapsulating the aqueous phase in the emulsion, thus converting the liquid aqueous phase into encapsulated aqueous beads, whereby a dispersion comprising aqueous beads is formed and the active ingredient or active ingredients are dissolved or incorporated in the aqueous beads, and

f) processing the dispersion obtained in step e) to form microcapsules where the encapsulated aqueous beads are further encapsulated by the solidified hydrophobic shell matrix.

A further aspect of the present invention relates to the use of the microcapsules of the present invention in food/feed industry and in pharmaceutical or cosmetic applications.

The invention is based on the concept of adding an encapsulating material, for example a hydrocolloid or any other suitable encapsulating material or mixture thereof, to the aqueous phase comprising the active ingredient(s) or to the hydrophobic phase in melted form, forming an emulsion of the aqueous phase and of the melted hydrophobic phase and, subsequently, encapsulating the active ingredient(s) in an aqueous bead or beads in the emulsion. The encapsulation of the aqueous phase is performed by gelling, cross-linking, coacervation, sintering or by any other suitable means. This results in dispersion where encapsulated aqueous beads comprising the active ingredient are dispersed in the hydrophobic phase. The dispersion is cooled below the melting or dropping point of the hydrophobic phase by any suitable process, which results in the formation of microcapsules. The cooling process can be performed, for example by spray cooling or fluidised bed cooling. The microcapsules comprise a number of encapsulated aqueous beads, which further contain the active ingredient(s), and the encapsulated aqueous beads are further encapsulated in or by a solidified hydrophobic shell matrix.

An advantage of the present invention is that the release of the active ingredient(s) from the microcapsules can be controlled. The release rate of a water-soluble active ingredient in a conventionally spray cooled fat matrix microcapsule is usually not controlled by the melting of the fat matrix but rather by the diffusion of water into the microcapsule and subsequent migration of the active ingredient outside the microcapsule. The release rate of the active ingredient from conventional spray cooled

microcapsules is usually very high. Typically, the release rates of the active ingredients are in the range of approximately 80% release within 15 minutes, depending on the nature of the encapsulated active ingredient. The novel and inventive microcapsules of the present invention have a much lower rate and/or sustained release of the active ingredients since most of the active ingredients are released when the solidified hydrophobic shell matrix is actually "melted". The release of the active ingredients from the microcapsules of the present invention can be controlled and the release can be initiated in various ways, for example by heat treatment, e.g. by heating, such as in a microwave oven, or by freezing, by stress treatment or by any other suitable process. The release of the active ingredients from the microcapsules of the present invention can also be sustained or it can happen very slowly.

Another advantage of the microcapsules of the present invention is that the stability of the microcapsules is improved. Since the active ingredients are dissolved or incorporated in encapsulated, preferably in gelled or cross-linked aqueous beads, which are further encapsulated in or by the solidified hydrophobic shell matrix, the aqueous phase is not able to migrate or evaporate to the shell matrix or outside the shell matrix.

An advantage of the microcapsules of the present invention compared to the microcapsules of the prior art, for example microcapsules prepared according to the cited references of Lee et al, Dinsmore et al, Mofidi et al or Wong et al, is that the hydrophobic phase is used to form a further encapsulation, thus forming microcapsules, where the active ingredient(s) is/are first encapsulated inside an aqueous bead and then further encapsulated in a hydrophobic phase.

The new improved properties of the microcapsules of the present invention enable the use of the microcapsules of the present invention in a wide variety of applications, for example in various applications in the food /feed or pharmaceutical fields.

Yet another advantage of the method of the invention is that it enables a high production capacity to be achieved while the costs are still low.

In the present specification in one aspect the term "encapsulated in or by the solidified hydrophobic shell matrix" may be taken to mean "encapsulated in the solidified hydrophobic shell matrix". In another aspect the term "encapsulated in or by the solidified hydrophobic shell matrix" may be taken to mean "encapsulated by the solidified

hydrophobic shell matrix".

For ease of reference, these and further aspects of the present invention are now discussed under appropriate section headings. However, the teachings under each section are not necessarily limited to each particular section.

Brief description of the drawings

In the following, the invention will be described in greater detail by means of preferred embodiments and with reference to the examples.

Mention of "WOK" in the Figures and the present specification is reference to samples in accordance with the invention.

Figure 1 is a graphical presentation of the results of Example 7. It illustrates the comparison between release rates of encapsulated and conventionally spray cooled calcium propionate.

Figure 2 is a transmitted light microscopy image of microcapsules in accordance with the invention

Figure 3 is ESEM pictures of microcapsules in accordance with the invention.

Figure 4 is a graph of a comparison of the release profiles of spray cooled, inventive and unencapsulated Ca^{2+} propionate:

Figure 5 is a graph of a comparison of the release profiles of spray cooled, inventive and unencapsulated citric acid.

Figure 6 is a graph of a comparison of the release profiles of spray cooled and nisin in accordance with the invention at 30C

Figure 7 is a graph of a comparison of the release profiles of betaine samples in accordance with the invention

Figure 8 is a transmitted light microscopy image of microcapsules in accordance with the invention that have been frozen, showing cracking of the fat particles due to expansion of inner aqueous phase upon crystallisation

Detailed description of the invention

The present invention relates to microcapsules which comprise a solidified hydrophobic shell matrix, an encapsulated aqueous bead or beads which is/are further encapsulated in or by the solidified hydrophobic shell matrix, and an active ingredient or active

ingredients dissolved or incorporated in the encapsulated aqueous bead or beads.

Preferably, the aqueous bead contains an encapsulating material, such as a hydrocolloid or any other suitable encapsulating material or mixture thereof in a concentration suitable to be susceptible to gelling, cross-linking, coacervation or sintering. Preferably, the encapsulated aqueous bead is a gelled or cross-linked hydrocolloid bead.

According to one aspect of the present invention, the active ingredient or active ingredients is/are double encapsulated in the microcapsules. First, the active ingredient is dissolved or incorporated in an aqueous phase containing encapsulating material, such as hydrocolloid or any other suitable encapsulating material or mixture thereof, and the aqueous phase is encapsulated, for example by gelling, cross-linking, coacervation, sintering or by any other suitable means, and the resulting encapsulated aqueous bead or beads is/are further encapsulated in a solidified hydrophobic shell matrix.

Hydrophobic Shell

The hydrophobic shell matrix is selected based on desired properties of the microcapsule, for example based on the intended use of the microcapsules, storage temperature, etc. Preferably the hydrophobic shell matrix should have a melting point above 45°C so that it can be stored at room temperature, in general any hydrophobic material can be used if the microcapsules are stored below the melting temperature of the hydrophobic material.

In this application, melted form means that the hydrophobic phase is at the lowest temperature at which the hydrophobic phase is sufficiently fluid to drip, as determined by test method ASTM D 566 or D 265.

The hydrophobic shell matrix or hydrophobic phase may be selected from the group comprising fats, oils, waxes, resins, emulsifiers or mixtures thereof, which are preferably food-grade. Preferably the hydrophobic shell matrix or hydrophobic phase is selected from the group comprising animal oils and fats, fully hydrogenated vegetable or animal oils, partially hydrogenated vegetable or animal oils, unsaturated, partially hydrogenated or fully hydrogenated fatty acids, unsaturated, partially hydrogenated or fully hydrogenated fatty acid monoglycerides and diglycerides, unsaturated, partially hydrogenated or fully hydrogenated esterified fatty acids of monoglycerides or

diglycerides, unsaturated, partially hydrogenated or fully hydrogenated free fatty acids, other emulsifiers, animal waxes, vegetable waxes, mineral waxes, synthetic waxes, natural and synthetic resins and mixtures thereof.

Animal oils and fats are such as, but not restricted to, beef tallow, mutton tallow, lamb tallow, lard or pork fat, sperm oil. Vegetable oils and in particular hydrogenated or partially hydrogenated vegetable oils are such as, but not restricted to, canola oil, cottonseed oil, peanut oil, corn oil, olive oil, soybean oil, sunflower oil, safflower oil, coconut oil, palm oil, linseed oil, tung oil and castor oil. Free fatty acids are such as, but not restricted to, stearic acid, palmitic acid and oleic acid. Other emulsifiers are such as, but not restricted to, polyglycerol esters, sorbitan esters of fatty acids. Animal waxes are such as, but not restricted to, beeswax, lanolin, shell wax or Chinese insect wax. Vegetable waxes are such as, but not restricted to, carnauba, candelilla, bayberry or sugarcane waxes. Mineral waxes are such as, but not restricted to, paraffin, microcrysalline petroleum, ozocerite, ceresin or montan. Synthetic waxes are such as, but not restricted to, low molecular weight polyolefin, polyol ether-esters and Fisher-Tropsch process synthetic waxes. Natural resins are such as rosin, balsam, shellac and zein.

Encapsulating Material

The encapsulated aqueous bead(s) in the microcapsules of the present invention contain encapsulating material, such as a hydrocolloid which is any food-grade hydrocolloid or any other suitable encapsulating material and which is susceptible to encapsulation by gelling, cross-linking, coacervation, sintering or by any other suitable means.

The encapsulating material may be selected from the group comprising hydrocolloids, shellac, zein, any synthetic or natural water-soluble polymers, any water-insoluble microparticles, such as silicone dioxide, titanium dioxide, synthetic or natural food-grade polymer beads or any water-insoluble solid particles which have a particle size substantially smaller than the size of the aqueous droplets in the aqueous phase and susceptible to sintering and mixtures thereof.

Preferably the hydrocolloid is selected from sodium alginate, gum arabic, gellan gum, starch, modified starch, guar gum, agar gum, pectin, amidified pectin, carrageenan, gelatine, chitosan, mesquite gum, hyaluronic acid, cellulose derivatives such as cellulose

acetate phtalate, hydroxy propyl methylcellulose (HPMC), methyl cellulose, ethyl cellulose and carboxy methyl cellulose (CMC), methyl acrylic copolymers, such as Eudragit®, psyllium, tamarind, xanthan, locust bean gum, xanthan/locust bean gum mixture, whey protein, soy protein, sodium caseinate, any food-grade protein and mixtures thereof.

In further aspects the encapsulating material may be selected from any mixture of oppositely charged hydrocolloids such as gelatine/arabic gum, gelatine/CMC, any proteins/ionic hydrocolloids, any combination of hydrocolloids and a solubility-reducing agent such as salts, sugars, acid or base, or sucrose acetate isobutyrate (SAIB), dammar gum, glyceryl esters of wood rosin, or mixtures thereof. In the case where fats, waxes or emulsifiers are used, they must differ from the hydrophobic matrix. Such mixtures are particularly preferred as coacervation encapsulating material.

The aqueous beads in the microcapsules of the present invention are encapsulated. In this application, encapsulation means gelling, cross-linking, coacervation, sintering or encapsulation by any other suitable means of encapsulating. Preferably, the aqueous beads in the microcapsules of the present invention contain a hydrocolloid and the beads are preferably either gelled or cross-linked.

According to a preferred embodiment of the present invention, a microcapsule comprises a solidified hydrophobic shell matrix, a gelled or cross-linked aqueous hydrocolloid bead or beads encapsulated in the solidified hydrophobic shell matrix, and an active ingredient or active ingredients dissolved or incorporated in the gelled or cross-linked aqueous hydrocolloid bead or beads.

The gelled hydrocolloids typically have a gelling temperature above room temperature. Examples of gelled hydrocolloids include carrageenan, gelatine, guar gum, agar gum, starch, modified starch and mixture of xanthan and locust bean gum, mixture of carrageenan and locust bean gum and mixture of any gelling hydrocolloids and other non-gelling hydrocolloids.

The cross-linking of the hydrocolloids is carried out by using cross-linking agents or by a variety of mechanisms. If the hydrocolloid is a protein or polysaccharide bearing amino groups, such as chitosan, acic, arabic gum or mesquite gum, it can be cross-linked by using dialdehydes, such as glutaraldehyde. If the hydrocolloid is a polysaccharide, such

as sodium alginate, gellan gum or pectin, it can be cross-linked with multivalent ions, such as calcium or magnesium. The cross-linking can also be carried out by other mechanisms, such as heating, pH adjustment, applying pressure or by enzymatic cross-linking. Proteins, for example, can be cross-linked by subjecting a protein to a high pressure, preferably from 2 to 200 or 5 to 200 bar, and/or by subjecting a protein to a temperature which is above the denaturation temperature of the protein. The temperature during the heating depends on the hydrocolloid to be cross-linked. The enzymatic cross-linking of proteins can be carried out for example with transglutamidase. Based on the hydrocolloid used, a person skilled in the art is able to decide which method of gelling or cross-linking is used.

The aqueous beads in the microcapsules of the present invention can be encapsulated by coacervation. The coacervation of the encapsulating material, such as hydrocolloid, is carried out by using any suitable coacervation process. The coacervation can be performed for example by adding salt(s), sugar(s), or other additives, which cause the phase separation of the encapsulation material, such as the hydrocolloid(s). The coacervation can also be performed by subjecting the emulsion to heating, cooling, pH change by adding acid or base, which cause the phase separation of the encapsulating material(s), such as the hydrocolloid(s). The deposition of the coacervated phase around the aqueous phase and at the interface between the hydrophobic matrix and the aqueous phase is spontaneous and driven by surface tension forces. The coacervate layer can afterwards be subjected to cross-linking or hardening by any suitable means, which are known to persons skilled in coacervation.

The encapsulating materials suitable for coacervation are selected form the group comprising shellac, zein, any synthetic or natural hydrophobic polymers, fats, emulsifiers, waxes, any mixture of oppositely charged hydrocolloids, such as gelatine/arabic gum, gelatine/CMC, any proteins/ionic hydrocolloids, any combination of hydrocolloids and a solubility-reducing agent such as salts, sugars, acids or bases, or sucrose acetate isobutyrate (SAIB), dammar gum and glyceryl esters of wood rosin or mixtures thereof.

Sintering means in this application that the micro particles are fused together to form a porous or non-porous film. The sintering of the encapsulating material is carried out by providing a suitable amount of solid, non-soluble micro particles, which have a particle size substantially smaller than the size of the aqueous droplets in the aqueous phase. The micro particles are for example such as silicone dioxide, titanium dioxide, synthetic

or natural food-grade polymer beads or any water-insoluble solid particles which have a particle size substantially smaller than the size of the aqueous droplets in the aqueous phase. The micro particles are then allowed to deposit spontaneously around the aqueous phase by subjecting the micro particles to temperatures above their sintering temperature or the glass transition temperature, thereby forming a continuous film of the micro particles.

Active Ingredient

The active ingredient or mixture of active ingredients (which may be dissolved or incorporated in the gelled, cross-linked, coacervated or sintered aqueous bead) can be any ingredient, preferably a hydrophilic food or pharmaceutical ingredient, and it is selected based on the desired use of the microcapsules. The active ingredient may be for example an inorganic or organic salt or acid, such as calcium propionate, propionic acid, sorbic acid, calcium sorbate, ascorbic acid, sodium chloride, fumaric acid, potassium sorbate, citric acid or sodium bicarbonate. The active ingredient can also be a flavouring agent, such as a pizza flavour or a coffee flavour, or the active ingredient can be an antimicrobial or a preservative agent, such as a bacteriocin (e.g. nisin or pediocin), natamycin, nutrient or vitamin, such as vitamin C or betaine. A mixture of any of the above mentioned ingredients can also be used in the microcapsules.

Preferably, the active ingredient is selected from the group comprising flavours, flavour enhancers, nutrients, vitamins, preservatives, leavening agents, micro organisms, acidulants, antioxidants, colours, enzymes, gases, thickeners and any other food or pharmaceutical ingredients. Suitable pharmaceutically active ingredients include antibiotics, antimicrobials, anti-inflammatory agents, analgesics, sedatives, hypnotics, anxiolytic agents, antihistamines, antiarrhythmics, antihypertensive agents, antiparkinson agents, hormones.

The microcapsules of the present invention may comprise approximately 1 to 100 aqueous beads encapsulated in the hydrophobic shell matrix, preferably 5 to 50 aqueous beads. The size of a microcapsule is approximately between 40 to 800 microns, preferably 100 to 150 microns. The size of one aqueous bead may be approximately between 0.1 to 20 microns, preferably 1 to 5 microns. The number as well as the size of aqueous beads encapsulated in the solidified hydrophobic shell matrix in the microcapsule may vary, depending on the intended use of the microcapsules. The size of

the microcapsules of the present invention may also vary depending on the intended use.

Method

The present invention also relates to a novel method for preparing the microcapsules of the present invention which method comprises the steps of

a) providing an aqueous phase comprising an active ingredient or active ingredients dissolved or incorporated in the aqueous phase,

b) providing a hydrophobic phase in melted form,

c) incorporating or dissolving an encapsulating material or mixture of encapsulating materials in the aqueous phase or in the hydrophobic phase,

d) combining the aqueous phase with the hydrophobic phase and homogenizing or mixing the combined phases to form a water-in-oil emulsion,

e) encapsulating the aqueous phase in the emulsion, thus converting the liquid aqueous phase into encapsulated aqueous beads, whereby a dispersion comprising aqueous beads is formed and the active ingredient or active ingredients are encapsulated in the aqueous beads, and

f) processing the dispersion obtained in step e) to form microcapsules where the encapsulated aqueous beads are further encapsulated in or by the solidified hydrophobic shell matrix.

The aqueous phase means in this application water or a mixture of water and any other water-miscible solvents, such as ethanol, ethylene glycol or glycerol. The aqueous phase may also contain additives, such as carbohydrates, such as monosaccharides or oligosaccharides to modify the properties of the hydrocolloid gel, inorganic salts to modify the properties of protein gels, preservatives to avoid deterioration of the microcapsules by bacteria or fungus or emulsifiers as processing aids, sorbitan tristearate or other emulsifiers as crystal form modifier, hydrophobic natural or synthetic polymers to modify mechanical properties of the matrix, plastizisers, preservatives to avoid deterioration of the microcapsules.

The combining of the aqueous phase with the hydrophobic phase is preferably performed by mixing.

The homogenisation in step d) is preferably performed by high-shear mixing or by in-line

mixing.

The encapsulating material is a hydrocolloid, a mixture of hydrocolloids or any other encapsulating material or mixture thereof.

The encapsulating in step e) may be performed by gelling, cross-linking, coacervation, sintering or by any other suitable encapsulating process which results in the encapsulation of the aqueous phase comprising the active ingredient or active ingredient.

The encapsulating by gelling in step e) may be performed by cooling the emulsion. The encapsulating materials suitable as gelling encapsulating materials may be selected from the group comprising carrageenan, gelatine, starch, modified starch, agar gum, guar gum and mixture of xanthan and locust bean gum or mixture of any gelling hydrocolloids.

The encapsulating by cross-linking in step e) is performed by using cross-linking agents or by a variety of mechanisms, such as heating, applying pressure or by enzymatic cross-linking. The cross-linking may be performed by subjecting the emulsion to heating at a temperature between 60 and 120°C. The cross-linking may also be performed by subjecting the emulsion to a pH value, which causes the denaturation of the hydrocolloids. The pH value is typically between 2 and 12. The cross-linking can also be performed by subjecting the emulsion to pressure between 2 to 200 bar.

The cross-linking agent may be selected from the group comprising dialdehydes, such as glutaraldehyde, divalent ions, such as calcium or magnesium or enzymes or other cross-linking compounds, such as irridoids.

The encapsulation by gelling or cross-linking results in the formation of microcapsules, in which the active ingredient is encapsulated in jelly-like beads formed from the hydrocolloid network, and these are then further encapsulated in the hydrophobic shell matrix.

The encapsulating by coacervation in step e) may be performed by reducing the solubility of the encapsulating material, such as the hydrocolloid, so that a coacervate phase is formed, and which coacervate phase further deposits itself around the aqueous phase. The aqueous phase in the emulsion is encapsulated forming a dispersion containing encapsulated solid aqueous beads.

The coacervation can be performed either by using a hydrophilic encapsulating material or by using a hydrophobic encapsulating material. If hydrophilic encapsulating material is used, the hydrophilic encapsulating material is first dissolved in the aqueous phase and a solubility-reducing process, such as a change in the temperature or pH or use of additives, is typically applied to bring the hydrophilic encapsulating material out of the aqueous phase, which is followed by the deposition of said encapsulating material at the interface between the hydrophobic phase in the melted form and the aqueous phase. After that the encapsulating material is optionally hardened by changing the temperature or pH or by adding additives. When hydrophobic encapsulating material is used, the hydrophobic encapsulating material is typically first dissolved in the hydrophobic phase in melted form and a solubility-reducing process, such as change in the temperature or adding additives, may be applied to bring the hydrophobic encapsulating material out of the hydrophobic phase. This may be followed by deposition of said encapsulating material at the interface between the hydrophobic phase and the aqueous phase.

The encapsulating by sintering in step e) may be performed by providing a suitable amount of solid, non-soluble micro particles, such as silicone dioxide, titanium dioxide, synthetic or natural food-grade polymer beads or any water-insoluble solid particles which have a particle size substantially smaller than the size of the aqueous droplets in the aqueous phase, and which micro particles are susceptible to sintering in the emulsion. After that the micro particles are allowed to deposit spontaneously around the aqueous phase at the interface between the hydrophobic phase and the aqueous phase and subjecting the micro particles to a temperature above their sintering temperature or their glass transition temperature. The micro particles are fused together to form a continuous film. A dispersion of aqueous beads encapsulated by a thin film of sintered micro particles in the hydrophobic shell matrix is thus formed.

The encapsulating materials suitable as sintering encapsulating materials may be selected form the group comprising any water-insoluble microparticles, such as silicone dioxide, titanium dioxide, synthetic or natural food-grade polymer beads or any water-insoluble solid particles which have a particle size substantially smaller than the size of the aqueous droplets in the aqueous phase in the hydrophobic matrix.

The encapsulation by coacervation or sintering results in microcapsules, where a thin coating of the encapsulating material is deposited around the aqueous beads comprising

active ingredient(s) and the bead or the beads is/are further encapsulated in or by the hydrophobic shell matrix.

The forming of the dispersion of the combined solution in step e) is performed by any suitable process or means which reduce the solubility of the dissolved encapsulating material resulting in the deposition of the encapsulating material around the aqueous phase. Preferably step e) is performed by a temperature change, either by decreasing or increasing the temperature, or by addition of additives.

The processing in step f) is carried out by any suitable method, which results in the solidification of the hydrophobic phase forming a hydrophobic shell matrix and the formation of the microcapsule. Preferably the processing is done by spray cooling or by fluidised bed cooling. Preferably the processing is done by spray cooling.

The hydrophobic phase is selected based on desired properties of the microcapsules, for example based on the intended use of the microcapsules, storage temperature, etc. The hydrophobic phase should preferably have a melting point above 45°C so that it can be easily stored at room temperature.

Preferably the present invention relates to a method which comprises the steps of
a) providing an aqueous phase comprising a hydrocolloid or a mixture of hydrocolloids and an active ingredient or active ingredients,
b) providing a hydrophobic phase in melted form,
c) combining the aqueous phase of step a) with the hydrophobic matrix of step b) and homogenizing the combined solution to form an emulsion,
d) gelling or cross-linking the hydrocolloids in the emulsion, whereby a dispersion comprising gelled or cross-linked hydrocolloid beads is formed and the active ingredient or active ingredients are dissolved or incorporated in the gelled or cross-linked hydrocolloid beads, and
e) cooling the dispersion obtained in step d) by spray cooling or fluidised bed cooling to form microcapsules where the gelled or cross-linked hydrocolloid beads are encapsulated in the solidified hydrophobic shell matrix.

The combining of the aqueous phase of step a) with the hydrophobic phase of step b) is preferably performed by mixing.

The homogenisation in step c) is preferably performed by high-shear mixing or by in-line mixing.

The hydrocolloid to be used in the present invention may be any food-grade hydrocolloid and is preferably water-soluble and susceptible to gelling and/or cross-linking.

The hydrocolloid comprised in the emulsion is preferably either gelled or cross-linked. The hydrocolloid(s) to be gelled should have a gelling temperature above the storage temperature. Examples of gelling hydrocolloids include carrageenan, gelatine, starch, modified starch, agar gum, guar gum and mixture of xanthan and locust bean gum or mixture of any gelling hydrocolloids and any other non-gelling hydrocolloids. The gelling of the hydrocolloids in the emulsion may be performed by the cooling of the emulsion, either before or during the cooling step. If the gelling of the hydrocolloid is carried out during the cooling, the emulsion is cooled after being formed. If the gelling of the hydrocolloid is carried out before the spray cooling, a dispersion is formed which comprises gelled hydrocolloid beads, and this dispersion is then cooled to form microcapsules.

The cross-linking agent may be selected from the group comprising dialdehydes, such as glutaraldehyde, divalent ions, such as calcium or magnesium or other cross-linking compounds, such as irridoids.

The cooling of the dispersion is preferably performed by spray cooling in a spray cooling tower or by fluidised bed cooling in a fluidised bed apparatus. During the spray cooling the hydrophobic matrix, which is in a melted form in the dispersion, is cooled so that it solidifies into particle form, encapsulating the hydrocolloid bead. Room temperature gas or cooled gas can be used in the cooling tower. Preferably the gas or the cooling gas is air. The temperature of the cooling gas may be between -270 and 50°C, preferably between -50 and 40°C and more preferably between -20 and 20°C.

The properties of the microcapsules can be altered by altering the process parameters of the above-described methods. For example, plastizisers can be added into the hydrophobic matrix phase to improve flexibility and to modify mechanical properties of the outer shell, lipase enzymes can be added in to the aqueous phase to modify the release rate.

One microcapsule prepared according to the method of the present invention may comprise approximately 1 to 100 aqueous beads embedded in the hydrophobic shell matrix, preferably 5 to 50 aqueous beads. The size of the microcapsule is typically approximately 40 to 800 microns, preferably 100 to 150 microns. The size of one aqueous bead is typically approximately 0.1 to 20 microns, preferably 1 to 5 microns.

The present invention also relates to the use of the microcapsules of the present invention. The microcapsules described above can be used in a wide variety of applications in food industry and in pharmaceutical applications.

The microcapsules of the present invention can be used in a great variety of applications, depending for example on the properties of the microcapsules, the active ingredient or a mixture thereof, the hydrocolloid, the hydrophobic matrix or the size of the microcapsules. A controlled release of the active ingredients from the microcapsules is achieved by the present invention. The release of the active ingredients from the microcapsules can be controlled by initiating the release in various ways, for example by heat treatment, by heating in a microwave oven, or by any other suitable process. The release of the active ingredients from the microcapsules of the present invention can also happen very slowly. The release of the ingredient also takes place upon freezing of the microcapsules. Freezing causes the water phase to expand, which causes the external hydrophobic matrix to crack. Upon thawing, the active ingredient is quickly released from the microcapsule.

In bakery, for example, delayed release of antimolding agent can be achieved with the microcapsules of the present invention. This is important in order to avoid inhibition of the required activity of the baker's yeast. If nisin or natamycin is used as the active ingredients, increased heat stability is achieved for example in pasteurised or heat-processed foods. Delayed release of sodium chloride is also very important for example in cheeses to avoid harmful interaction with starting cultures. Thermal stability of vitamin C in bakery/confectionery can be achieved with the microcapsules of the present invention.

Betaine is used in feed to supplement to nutrient intake of fish and shrimps. However, as it is very hygroscopic and highly water-soluble, it is difficult to ensure consistent delivery to fish over extended period of time because the betaine leaches from the feed pellets before they are eaten by the animals. Encapsulation in accordance with the present

invention may prevent early dissolution, thus ensuring effective delivery of betaine to fish. Spray cooling cannot, produce microcapsules that retain their content in aqueous media for more than 10-15 minutes, which is not long enough for the fish feed application. These problems are addressed by betaine encapsulated in accordance with the present invention.

The present invention relates to the use of microcapsules as flavours, bacteriocin agents, preservative agents and agents providing slow, controlled and/or sustained release of the active ingredient(s). The microcapsules of the present invention may be used in a wide variety of pharmaceutical applications where slow, controlled and/or sustained release of the pharmaceutically active ingredient is required. Such uses include for example depot-tablets and trans-dermal application systems.

Controlled release of flavours in food products, such as baked goods, pizza, instant coffee, tea bags, is achieved with the microcapsules of the present invention containing flavours as the active ingredient. The encapsulated flavours are retained in the product until heat and/or stress treatment is applied to release the flavours. Heat can be provided for example by a microwave oven, conventional oven or hot water. Stress can be provided for example by processing conditions or mastication.

Slow release of bacteriocin for example in processed meat products or in beverages, such as orange juice, is achieved with the microcapsules of the present invention. If a preservative agent is used as an active ingredient in the microcapsules of the present invention, the preservative agent is slowly released in the product as it is naturally degraded. This effectively prevents growth of fungi or other undesirable micro-organisms for a longer period of time than a non-encapsulated preservative, thus ensuring a longer shelf life for the food product. The coating can also provide thermal stability to bacteriocin and preservative agents so as to survive heat treatment and harsh processing conditions, but to remain active during storage of the processed product.

The microcapsules of the present invention provide delayed release of salt in cheese production, which allows a 1-step process instead of a 2-step process. The delayed release of salt allows the starter culture to work properly at the beginning without being subjected to the detrimental effect of salt. When fermentation is over, the salt is released. In a typical process, salt is added after fermentation by time-consuming dipping of the cheese in brine.

Delayed release of an anti-microbial agent in bakery applications is achieved by the microcapsules of the present invention. The preservatives are widely used to extend the shelf life of breads and other bakery products, but at the expense of detrimentally affecting the effectiveness of the yeast. The delayed release allows a more efficient use of the yeast, while also providing the preservative properties after the active ingredient is released during baking. As an added benefit, propionic acid, which is much more potent than its calcium salt but much more difficult to handle due to its high acidity and liquid form, can be transformed into a stable powder which is easy to handle.

The pharmaceutically active ingredients encapsulated according to the present invention provide slow, controlled and/or sustained release of the active ingredient over time, for example in depot-tablets, in a much cheaper way compared to how it is performed today (by fluidised bed coating). The encapsulation according to the present invention also provides stability of the pharmaceutically active ingredients in the gastric tract (low pH), which enables them to be released later on in the intestinal tract where most of the pharmaceutically active ingredients are actually absorbed. Examples of suitable pharmaceutically active ingredients include antibiotics, antimicrobials, anti-inflammatory agents, analgesics, sedatives, hypnotics, anxiolytic agents, antihistamines, antiarrhythmics, antihypertensive agents, antiparkinson agents and hormones.

Other ingredients and possible applications include
Betaine - Feedstuffs
Nisin - Bakery
Natamycin - Bakery
Ascorbic acid - Bakery, rolled dough
Sorbic acid - Bakery
Flavours - Frozen pizza, beverages, cereals
Propionic acid - Bakery
Nisin - Sausage casing, vinaigrette
Citric, fumaric - Bakery, tortillas
Water - Low - fat spread, bakery
Sodium chloride is used in cheese as a flavouring agent and is usually added at the end of the ripening process by immersing the whole cheese in brine. This process is time-consuming and costly. Initial tests showed that encapsulated sodium chloride could be added to the milk at the beginning of the fermentation process and the delayed release

decreased the detrimental effect of the salt on the starter culture but still ensure proper flavouring at the end of the ripening.

Ascorbic acid is used in laminated dough to strengthen the gluten network through oxidative crosslinking of the proteins. Crosslinking and strengthening of the dough matrix should take place after the mixing/laminating stage of dough processing so as to not impair the structure of the final product. Premature crosslinking and strengthening results in a dough that is difficult to work and visually unappealing. Delaying the release of the ascorbic acid by encapsulation would allow the dough to be easily processed before the strengthening occurs. Encapsulation has the advantages over spray cooling of more effectively delaying the release and providing the ascorbic acid already dissolved, should ensure rapid distribution of the acid after it is released.

Water can also benefit from encapsulation. The present invention is capable of tightly encapsulating water. Ciabatta bread with large and uneven pores may be produced with encapsulated water: the baking process would release the water droplets that would create large pores upon vaporisation. No-spattering low-fat spread could be achieved if the water used in the emulsion was encapsulate and less likely to immediately vaporise upon heating.

ADDITIONAL COMPONENTS

The encapsulated anti-microbial material may contain one or more components in addition to the core of active ingredient and the shell of encapsulating material. These one or more additional components may or may not be encapsulated within or by the shell together with the active ingredient. In other words the additional components may be encapsulated within or by the shell together with the active ingredient or may be "outside" the shell. When one or more additional components are provided a combination of the above is envisaged (one component may be within the shell and another component outside the shell).

Typically the encapsulated antimicrobial material will not be introduced into the foodstuff alone. Thus in one aspect the encapsulated antimicrobial material is introduced into the foodstuff in a carrier. Preferably the carrier is or comprises brine.

The density of the encapsulated antimicrobial material should match the density of the carrier (such as brine) to avoid separation or sedimentation of the encapsulated antimicrobial material, preventing even distribution of encapsulated antimicrobial material

during injection or tumbling. Thus in a preferred aspect the carrier and the encapsulated antimicrobial material have substantially the same density.

Matching the density of the carrier and the encapsulated antimicrobial material may be achieved by careful selection of carrier and encapsulated antimicrobial material. Alternatively it may be achieved by modification of the encapsulated antimicrobial material to have substantially the same density as the carrier, or by modification of the carrier to have substantially the same density as the encapsulated antimicrobial material. The encapsulated antimicrobial material may be modified by contacting the encapsulated antimicrobial material with oil, such as a brominated oil. The carrier may be modified by inclusion of an additional component such as xanthum gum.

The carrier may contain one or more additional components. However, in some aspects the carrier contains no additional components or contains no additional components that materially affect the properties of the composition.

In one preferred aspect the carrier further comprises an emulsifier. Preferably the emulsifier is selected from polyoxy-ethylene sorbitan esters (E432-E436) otherwise known as polysorbates (e.g. Tween 80, Tween 20), monoglycerides, diglycerides, acetic acid esters of mono-diglycerides, tartaric acid esters of mono-diglycerides and citric acid esters of mono-diglycerides.

The encapsulated anti-microbial material may contain one or more additional components. However, in some aspects the encapsulated anti-microbial material contains no additional components or contains no additional components that materially affect the properties of the composition.

In one preferred aspect the encapsulated antimicrobial material further comprises an extract obtained from or obtainable from a plant of the Labiatae family. Optionally in this aspect and particularly when the antimicrobial material consists of nisin, the composition comprises carvacrol in an amount of less than 0.075wt.% based on the composition and carvone in an amount of less than 15wt.% based on the composition. Compositions comprising an antimicrobial material and an extract obtained from or obtainable from a plant of the Labiatae family are discussed in our British Patent Application No. 0323335.0 Each of the teachings of GB 0323335.0 are applicable to the present system.

In this aspect preferably the extract obtained from or obtainable from a plant of the Labiatae family is not encapsulated within or by the shell together with the anti-microbial material.

In one preferred aspect the extract contains carvacrol in an amount of less than 0.075wt.% based on the composition, preferably in an amount of less than 0.04wt.% based on the composition, more preferably in an amount of less than 0.02wt.% based on the composition.

In one preferred aspect the extract contains carvone in an amount of less than 0.075wt.% based on the composition, preferably in an amount of less than 0.04wt.% based on the composition, more preferably in an amount of less than 0.02wt.% based on the composition.

In one preferred aspect the extract contains thymol in an amount of less than 0.1wt.% based on the composition, preferably in an amount of less than 0.075wt.% based on the composition, more preferably in an amount of less than 0.0wt.% based on the composition.

In one aspect the extract used is obtained from a plant of the Labiatae family.

It will be appreciated by one skilled in the art that by the term "extract" or "extracts" it is meant any constituent of the plant which may be isolated from the whole plant.

In one aspect the extract used in the present invention is obtainable from a plant of the Labiatae family. It will be appreciated by one skilled in the art that an extract obtainable from a plant may be obtained from a plant or may be isolated from the plant, identified and then obtained from an alternative source, for example by chemical synthesis or enzymatic production. For example the extract may be produced by a eukaryotic or prokaryotic fermentation, by a process of genetic manipulation. The present applicant have recognised that products present in a plant of the Labiatae family may synergistically increase the activity of a an antimicrobial material, preferably a bacteriocin. These products may be obtained from any source and will fall within the scope of the present invention.

The invention comprises use of a combination of a bacteriocin such as nisin and of the

Labiatae plant family, such as rosemary (*Rosmarinus officinalis*) or sage (*Salvia officinalis*) that together give enhanced control of Gram-positive bacteria in a food system. The extracts responsible for synergy in the present invention preferably refer to extracts of the plant family Labiatae that have been selectively extracted ("deodorised extracts") to increase their phenolic diterpene content (such as carnosol and carnosic acid), phenolic tripterpene content (such as ursolic acid, betulinic acid and oleanolic acid) or rosmarinic acid content. These deodorised extracts can be distinguished by their high phenolic diterpene content (for example greater than 3.5 wt.%) and their low level (less than 1 wt.%) of flavour-inducing compounds from plant essential oils and oleoresins that are used as flavours or fragrances. Essential oils are typically extracted by simple steam distillation of the plant material.

In one preferred aspect the extract is a deodorised extract. Preferably the (deodorised) extract contains from 1.0 to 70 wt.% phenolic diterpenes, preferably 3.5 to 70 wt.% phenolic diterpenes and less than 1 wt.% essential oil.

In one preferred aspect the extract is selected from phenolic diterpenes, phenolic triterpenes and rosmarinic acid.

In one preferred aspect the extract is or comprises a phenolic diterpene. Preferably the phenolic diterpene is selected from carnosic acid, carnosol, methylcarnosic acid and mixtures thereof. Preferably the phenolic diterpene is selected from carnosic acid and carnosol.

In one preferred aspect the extract contains phenolic diterpenes in an amount of greater than 1.0wt.% , based on the composition, preferably in an amount of is greater than 2.0wt.%, based on the composition, more preferably in an amount of is greater than 3.0wt.%, based on the composition, more preferably in an amount of is greater than 3.5wt.%, based on the composition.

In one highly preferred aspect the extract contains one or more phenolic triterpenes. Preferably the phenolic triterpenes are selected from betulinic acid, oleanolic acid, and ursolic acid.

In one preferred aspect is or comprises a phenolic triterpene. Preferably the phenolic triterpene is selected from betulinic acid, oleanolic acid, and ursolic acid.

In one preferred aspect the extract is or comprises rosmarinic acid.

In one preferred aspect the plant of the Labiatae family is selected from rosemary, sage, oregano, marjoram, mint, balm, savoury and thyme. In one preferred aspect the plant of the Labiatae family is selected from rosemary, sage, oregano, marjoram, mint, balm, and savoury. It will be understood that these name cover all species and varieties of plants known by these names.

In one preferred aspect the plant of the Labiatae family is selected from rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.) oregano (*Origanum vulgare* L.), marjoram (*Origanum marjorana* L.), mint (*Mentha* spp.), balm (*Melissa officinalis* L.), savoury (*Satureia hortensis*), thyme (*Thymus vulgaris* L.).

In one preferred aspect the plant of the Labiatae family is selected from rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.) oregano (*Origanum vulgare* L.), marjoram (*Origanum marjorana* L.), mint (*Mentha* spp.), balm (*Melissa officinalis* L.), and savoury (*Satureia hortensis*).

In one preferred aspect the plant of the Labiatae family is rosemary.

In one preferred aspect the extract contains flavour-inducing compounds and/or essential oils in an amount of less than 1 wt.% based on the extract. In one preferred aspect the extract contains flavour-inducing compounds and/or essential oils in an amount of less than 1 wt.% based on the composition.

Typically flavour-inducing compounds and/or essential oils are camphor, verbenone, borneol and alfa-terpineol.

In one preferred aspect the combined amount of camphor present in the extract is less than 1 wt.% (preferably less than 0.2 wt.%, more preferably less than 0.15wt.%, more preferably less than 0.1wt.%) based on the extract.

In one preferred aspect the combined amount of verbenone present in the extract is less than 1 wt.% (preferably less than 0.2 wt.%, more preferably less than 0.15wt.%, more preferably less than 0.1wt.%) based on the extract.

In one preferred aspect the combined amount of borneol present in the extract is less than 1 wt.% (preferably less than 0.2 wt.%, more preferably less than 0.15wt.%, more preferably less than 0.1wt.%) based on the extract.

In one preferred aspect the combined amount of alfa-terpineol present in the extract is less than 1 wt.% (preferably less than 0.2 wt.%, more preferably less than 0.15wt.%, more preferably less than 0.1wt.%) based on the extract.

In one preferred aspect the combined amount of camphor, verbenone, borneol and alfa-terpineol present in the extract is less than 1 wt.% (preferably less than 0.2 wt.%, more preferably less than 0.15wt.%, more preferably less than 0.1wt.%) based on the extract.

In one preferred aspect the encapsulated antimicrobial material further comprises a chelator. Preferably the chelator is selected from EDTA, citric acid, monophosphates, diphosphates, triphosphates and polyphosphates.

Further suitable chelator are taught in US 5573801 and include carboxylic acids, polycarboxylic acids, amino acids and phosphates. In particular, the following compounds and their salts may be useful:

Acetic acid, Adenine, Adipic acid, ADP, Alanine, B-Alanine, Albumin, Arginine, Ascorbic acid, Asparagine, Aspartic acid, ATP, Benzoic acid, n-Butyric acid, Casein, Citraconic acid, Citric acid, Cysteine, Dehydracetic acid, Desferri-ferrichrysin, Desferri-ferrichrome, Desferri-ferrioxamin E, 3,4-Dihydroxybenzoic acid, Diethylenetriaminepentaacetic acid (DTPA), Dimethylglyoxime, O,O-Dimethylpurpurogallin, EDTA, Formic acid, Fumaric acid, Globulin, Gluconic acid, Glutamic acid, Glutaric acid, Glycine, Glycolic acid, Glycylglycine, Glycylsarcosine, Guanosine, Histamine, Histidine, 3-Hydroxyflavone, Inosine, Inosine triphosphate, Iron-free ferrichrome, Isovaleric acid, Itaconic acid, Kojic acid, Lactic acid, Leucine, Lysine, Maleic acid, Malic acid, Methionine, Methylsalicylate, Nitrilotriacetic acid (NTA), Ornithine, Orthophosphate, Oxalic acid, Oxystearin, B-Phenylalanine, Phosphoric acid, Phytate, Pimelic acid, Pivalic acid, Polyphosphate, Proline, Propionic acid, Purine, Pyrophosphate, Pyruvic acid, Riboflavin, Salicylaldehyde, Salicyclic acid, Sarcosine, Serine, Sorbitol, Succinic acid, Tartaric acid, Tetrametaphosphate, Thiosulfate, Threonine, Trimetaphosphate, Triphosphate, Tryptophan, Uridine diphosphate, Uridine triphosphate, n-Valeric acid, Valine, and

Xanthosine

Many of the above sequestering agents are useful in food processing in their salt forms, which are commonly alkali metal or alkaline earth salts such as sodium, potassium or calcium or quaternary ammonium salts. Sequestering compounds with multiple valencies may be beneficially utilised to adjust pH or selectively introduce or abstract metal ions e.g. in a food system coating. Additional information chelators is disclosed in T. E. Furia (Ed.), CRC Handbook of Food Additives, 2nd Ed., pp. 271-294 (1972, Chemical Rubber Co.), and M. S. Peterson and A. M. Johnson (Eds.), Encyclopaedia of Food Science, pp. 694-699 (1978, AVI Publishing Company, Inc.) which articles are both hereby incorporated by reference.

The terms "chelator" is defined as organic or inorganic compounds capable of forming co-ordination complexes with metals. Also, as the term " chelator" is used herein, it includes molecular encapsulating compounds such as cyclodextrin. The chelator may be inorganic or organic, but preferably is organic.

Preferred chelator are non-toxic to mammals and include aminopolycarboxylic acids and their salts such as ethylenediaminetetraacetic acid (EDTA) or its salts (particularly its di- and tri-sodium salts), and hydrocarboxylic acids and their salts such as citric acid. However, non-citric acid and non-citrate hydrocarboxylic acid chelators are also believed useful in the present invention such as acetic acid, formic acid, lactic acid, tartaric acid and their salts.

As noted above, the term "chelator" is defined and used herein as a synonym for sequestering agent and is also defined as including molecular encapsulating compounds such as cyclodextrin. Cyclodextrins are cyclic carbohydrate molecules having six, seven, or eight glucose monomers arranged in a donut shaped ring, which are denoted alpha, beta or gamma cyclodextrin, respectively. As used herein, cyclodextrin refers to both unmodified and modified cyclodextrin monomers and polymers. Cyclodextrin molecular encapsulators are commercially available from American Maize-Products of Hammond, Ind. Cyclodextrin are further described in Chapter 11 entitled, "Industrial Applications of Cyclodextrin", by J. Szejtli, page 331-390 of Inclusion Compounds, Vol. III (Academic Press, 1984) which chapter is hereby incorporated by reference.

Preferably the chelator enhances the antimicrobial activity and/or antimicrobial spectrum

of the bacteriocin. More preferably the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin in respect of Gram-negative bacteria and other micro-organisms.

We have found that the provision of a chelator is particularly effective in view of the enhancement of the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin provided. This enhancement is possible irrespective of the manner in which the encapsulated antimicrobial material is delivered or the nature of the shell of the encapsulated antimicrobial material

Examples

Example 1 – Encapsulation of pizza flavour

First, a solution of 1.5 g κ-carrageenan in 110 ml of water is prepared at 85°C. To this is added 110 ml of a pre-heated (80°C) water-soluble liquid pizza flavour. The resulting mixture is thoroughly mixed. Secondly, a mixture of 200 g of a vegetable triglyceride (GRINDSTED ® PS 101, m.p. 58°C) and 11 g of acetylated emulsifier (Acetem 50 00) is melted at 85°C in a water bath. The melted fat mixture is kept under homogenisation (Silverson mixer, 8000 rpm) as the aqueous mixture is slowly incorporated. The homogenisation is maintained for 5 minutes after the whole aqueous mixture is added and then a solution of 0,45 g of polysorbate 80 in 15 ml of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature 10°C, outlet air temperature 28°C, rotating atomization wheel speed 10 000 rpm. A pizza-smelling free flowing powder is obtained.

A 6" frozen model pizza is sprinkled 1.5 g of the flavouring powder and baked in the microwave for 2 minutes at medium-high intensity. The flavoured pizza samples have a distinctly stronger pizza aroma when exiting the microwave compared to control pizza samples.

Example 2 – Encapsulation of coffee flavour

First, a solution of 1.5 g κ-carrageenan in 110 ml of water is prepared at 85°C. To this is added 110 ml of a pre-heated (80°C) water-soluble coffee flavour. The resulting mixture

is thoroughly mixed. Secondly, a mixture of 200 g of a vegetable triglyceride (GRINDSTED ® PS 101, m.p. 58°C) and 11 g of acetylated emulsifier (Acetem 50 00) is melted at 85°C in a water bath. The melted fat mixture is kept under homogenisation (Silverson mixer, 8 kRPM) as the aqueous mixture is slowly incorporated. The homogenisation is maintained for 5 minutes after the whole aqueous mixture is added and then a solution of 0.45 g of polysorbate 80 in 15 ml of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature 10°C, outlet air temperature 28°C, rotating atomization wheel speed 10 000 rpm. A coffee-smelling free flowing powder is obtained.

The flavouring powder is added to hot water (90°C) and a strong coffee aroma evolves within one minute.

Example 3. – Encapsulation of nisin

First, a solution of 15 g κ-carrageenan in 1000 ml of phtalate buffer at pH 3.5 is prepared at 85°C. To this is added 300 g of commercial nisin extract (Nisaplin®, Danisco). The resulting mixture is thoroughly mixed. At the same time, a mixture of 1333 g of a vegetable triglyceride (GRINDSTED ® PS 101, m.p. 58°C) and 73 g of acetylated emulsifier (Acetem 50 00) is melted at 85°C in a water bath. The melted fat mixture is kept under homogenisation (Silverson mixer, 8000 rpm) as the aqueous mixture is slowly incorporated. The homogenisation is maintained for 5 minutes after the whole aqueous mixture is added and then a solution of 3 g of polysorbate 80 in 40 ml of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature 10°C, outlet air temperature 28°C, rotating atomization wheel speed 10 000 rpm. A free flowing powder is obtained. The incorporation of encapsulated nisin in a suspension media for subsequent spraying onto food products such as sausages, sausage casings, meat products or any other food products requiring bactericides results in a much more stable nisin formulation compared to when unencapsulated or conventionally spray cooled nisin is used in the suspension media, thus dramatically improving survival rate of nisin until the pasteurisation of the food product. For example, spray cooled nisin is released in the suspension media, thus subjecting it to rapid degradation, at a rate of 57% after 3 days in the suspension media. The encapsulated nisin, as presented in this example, is released at a rate of only 7% after three days.

Example 4. – Encapsulation of nisin

First, a solution of 15 g sodium alginate in 1000 ml of phtalate buffer at pH 3.5 is prepared at 85°C. To this is added 300 g of commercial nisin extract (Nisaplin®, Danisco). The resulting mixture is thoroughly mixed. At the same time, a mixture of 1333 g of a vegetable triglyceride (GRINDSTED ® PS 101, m.p. 58C) and 73 g of acetylated emulsifier (Acetem 50 00) is melted at 85°C in a water bath. The melted fat mixture is kept under homogenisation (Silverson mixer, 8 kRPM) as the aqueous mixture is slowly incorporated. Following the incorporation of the aqueous mixture, a solution of 7 g of calcium chloride in 70 ml of water is added dropwise. The homogenisation is maintained for another 5 minutes and then a solution of 3 g of polysorbate 80 in 40 ml of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature 10°C, outlet air temperature 28°C, rotating atomization wheel speed 10 000 rpm. A free flowing powder is obtained. As mentioned previously, encapsulated nisin as presented in this example is much more stable in aqueous environment than a conventionally spray-cooled sample. For example, spray cooled nisin is released in the suspension media, thus subjecting it to rapid degradation, at a rate of 57% after 3 days in the suspension media. The encapsulated nisin, as presented in this example, is released at a rate of only 0,1% after three days.

Example 5. – Encapsulation of sodium chloride

First, a solution of 15 g κ-carrageenan in 1000 ml of water is prepared at 85°C. To this is added 585 g of sodium chloride. The resulting mixture is thoroughly mixed. At the same time, a mixture of 1333 g of a vegetable triglyceride (GRINDSTED ® PS 101, m.p. 58°C) and 73 g of acetylated emulsifier (Acetem 50 00) is melted at 85°C in a water bath. The melted fat mixture is kept under homogenisation (Silverson mixer, 8000 rpm) as the aqueous mixture is slowly incorporated. The homogenisation is maintained for 5 minutes after the whole aqueous mixture is added and then a solution of 3 g of polysorbate 80 in 40 ml of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature 10°C, outlet air temperature 28°C, rotating atomization wheel speed 10 000 rpm. A free flowing powder is obtained.

Example 6. – Encapsulation of sorbic acid

First, a solution of 15 g κ-carrageenan in 1000 ml of water is prepared at 85°C. To this is added 300 g of sorbic acid. The resulting mixture is thoroughly mixed. At the same time, a mixture of 1333 g of a vegetable triglyceride (GRINDSTED ® PS 101, m.p. 58°C) and 73 g of acetylated emulsifier (Acetem 50 00) is melted at 85°C in a water bath. The melted fat mixture is kept under homogenisation (Silverson mixer, 8000 rpm) as the aqueous mixture is slowly incorporated. The homogenisation is maintained for 5 minutes after the whole aqueous mixture is added and then a solution of 3 g of polysorbate 80 in 40 ml of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature 10°C, outlet air temperature 28°C, rotating atomization wheel speed 10 000 rpm. A free flowing powder is obtained.

Example 7 - Encapsulation of calcium propionate

First, a solution of 15 g κ-carrageenan in 1000 ml of water is prepared at 85°C. To this is added 300 g of calcium propionate. The resulting mixture is thoroughly mixed. At the same time, a mixture of 1333 g of a vegetable triglyceride (GRINDSTED ® PS 101, m.p. 58°C) and 73 g of acetylated emulsifier (Acetem 50 00) is melted at 85°C in a water bath. The melted fat mixture is kept under homogenisation (Silverson mixer, 8000 rpm) as the aqueous mixture is slowly incorporated. The homogenisation is maintained for 5 minutes after the whole aqueous mixture is added and then a solution of 3 g of polysorbate 80 in 40 ml of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature 10°C, outlet air temperature 28°C, rotating atomization wheel speed 10 000 rpm. A free flowing powder is obtained. The release rate of the calcium propionate is determined using the basket method. The curve is shown in Figure 1.

Example 8 - Encapsulation of propionic acid

First, a solution of 40 g of amidified low ester pectin (Danisco Pectin 2580) in 750 ml of water is prepared at 85°C. To this is added 250 g of propionic acid. The resulting mixture is thoroughly mixed. At the same time, a mixture of 1333 g of a vegetable triglyceride (GRINDSTED ® PS 101, m.p. 58°C) and 73 g of acetylated emulsifier (Acetem 50 00) is

melted at 85°C in a water bath. The melted fat mixture is kept under homogenisation (Silverson mixer, 8000 rpm) as the aqueous mixture is slowly incorporated. Following the incorporation of the aqueous mixture, a solution of 5 g of calcium chloride in 30 ml of water is added dropwise. The homogenisation is maintained for another 5 minutes and then a solution of 3 g of polysorbate 80 in 40 ml of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature 10°C, outlet air temperature 28°C, rotating atomization wheel speed 10 000 rpm. A free flowing powder is obtained.

Example 8

Material and equipment

The details of all the raw materials use in the processing of samples in accordance with the present invention are given below. The phosphoric acid buffer was prepared by dissolving 22 mmol of phosphoric acid in approx. 1,8 L of tap water, adjusting the pH to 3 by adding 1M NaOH and completing to exactly 2 L.

Material	function	Comments
PS101	shell material	triglyceride, m.p.
Carrageenan 100		pure κ
Alginate FD175		
Gelatin		Isoelectric point: 8.5
pectin 1400		high ester
pectin 2580		Amidified
Agar NQS 200		
Xanthan		
LBG 246 CAP		
phosphoric acid	Buffer	
acetem 50	Stabilise the w/o emulsion	HLB: 1,5
polysorbate 80		HLB: 15,4

The mixing/emulsion step was performed with a Silverson Mixer L4R-T (Waterside-Chestam-Bucks, England) at 6000 rpm using a round-hole emulsion head. The final

spray cooling step of all the experiments in accordance with the invention was done on a pilot-scale NIRO spray tower (Niro, Denmark) equipped with a rotating atomization wheel. The inlet and outlet air temperatures were usually 10 and 30 C respectively. The atomization wheel was run at 8000 rpm. The feed flow into the tower was regulated manually to reach the set outlet air temperature. The NMR experiments were done by Pulse Gradient Spin Echo experiment on a GU200 (Bruker, Germany)

Release method

The release test is based on a standard dissolution test (USP 27, Method 711, Apparatus 1) used in the pharmaceutical industry to measure the rate at which active ingredients dissolve from the dosage form (e.g. tablet, capsule). In the release test, a small quantity of encapsulated ingredient is placed in a wire basket, which is then immersed in water and rotated. As the basket rotates, the encapsulated ingredient is released and the amount of free ingredient in the dissolution media correspondingly increases. Depending on the nature of the ingredient encapsulated, the rate of dissolution can be measured by following the change in pH, ion concentration (specific or general) or through sampling and subsequent HPLC analysis. The amount of ingredient in the dissolution media is measured at intervals over the 60-minute test period and is reported in terms of the 'normalised' concentration (that is the concentration proportional to the theoretical concentration, based on encapsulation payload). From this data, a release curve showing the increase in normalised concentration against elapsed time can be constructed (see figure 4 for an example).

Example 8.1 - Gelled hydrocolloids

First, 15g of gelling hydrocolloid(s) is added to 1000mL of water and the subsequent solution heated to 85°C. The pre-warmed (40-60 C) ingredients to be encapsulated are added to the hydrocolloid solution with constant mixing. At the same time, a mixture of 1333 g of a vegetable triglyceride (GRINSTED ® PS 101, m.p. 58°C) and 73 g of acetylated emulsifier (Acetem 50 00) is melted at 85°C in a water bath. The melted fat mixture is mixed with a high shear mixer (Silverson mixer, 8000 rpm) as the aqueous mixture is slowly added. The homogenization is maintained for 5 minutes after all the aqueous mixture has been added before a solution of 3 g of polysorbate 80 in 40 ml of water is added, also under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled, typically in a Niro spray tower with the

following parameters: inlet air temperature: 0-10°C, outlet air temperature 25-35°C, rotating atomization wheel speed: 10000 rpm.

Example 8.2 - Crosslinked hydrocolloids

A solution of crosslinkable hydrocolloid(s) in 1000 mL of water is prepared at 85°C. To this is added the pre-warmed (40-60C) ingredients to be encapsulated. The resulting mixture is thoroughly mixed. At the same time, a mixture of 1333 g of a vegetable triglyceride (GRINSTED ® PS 101, m.p. 58C) and 73 g of acetylated emulsifier (Acetem 50 00) is melted at 85°C in a water bath. The melted fat mixture is kept under homogenisation (Silverson mixer, 8 kRPM) as the aqueous mixture is slowly incorporated. Following the incorporation of the aqueous mixture, a solution of 7 g of calcium chloride in 70 ml of water is added dropwise. The homogenisation is maintained for another 5 minutes and then a solution of 3 g of polysorbate 80 in 40 ml of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature: 0-10°C, outlet air temperature 25-35°C, rotating atomization wheel speed: 10000 rpm.

Samples

The active ingredients, coating materials and encapsulation recipes listed above were combined to produce as series of different encapsulated food ingredients. The table below summarises the details of the samples produced and tested.

Active Ingredient	Payload w/w %	Aquous phase	Hydrocolloids	Recipe
pizza flavour	31	water	carrageenan	Ex 8.1

Active Ingredient	Payload w/w %	Aquous phase	Hydrocolloids	Recipe
nisin	13	phosphoric buffer (pH 3.5)	carrageenan	Ex 8.1
	13		alginate	Ex 8.2
betaine	29	water	carrageenan	Ex 8.1
			alginate	Ex 8.2
			gelatine	Ex 8.1
			pectin 1400	Ex 8.1
			pectin 2580	Ex 8.2
			agar	Ex 8.1
			LBG/Xanthan	Ex 8.1
			LBG/Carrageenan	Ex 8.1
Ca^{2+} propionate	11	water, pH 8,5	carrageenan	Ex 8.1
citric acid	24	water, pH 3,0	pectin 2580	Ex 8.2
fumaric acid	24	water, pH2,8	pectin 2580	Ex 8.2

The particular advantages of encapsulate the above ingredients and the background of the applications are explained below.

Calcium propionate: Spray cooled calcium propionate is a commercial product (PRO45) used in bakery as a preservative. Encapsulation prevents the detrimental interaction of the propionate with yeast during the first 15-20 minutes of the mixing/proofing stage. The delayed release of propionate results in an appropriate shelf life and savings on yeast compared to unencapsulated calcium propionate. Use of an encapsulated product in accordance with the present invention as an alternative to PRO45 would have the advantage of 1) delivering the propionate already dissolved, which may reduce the browning occurring sometimes because of extreme pH "hotspots" and 2) possible slower release rate, which would allow further saving on yeasts.

Citric acid is to lower the pH of bakery goods such as tortillas and acts as a preservative where other organic acids, such as fumaric acid, are not permitted.

Nisin is a powerful antimicrobial used in many applications to extend shelf life and prevent spoilage by micro-organisms. However, it is rapidly degraded at neutral/basic pH and as a protein, is inherently unstable at high temperature. An encapsulated nisin in

accordance with the present invention may show improved thermal stability, and additional controlled release functionality, necessary to prevent excessive degradation during food processing. Possible applications include processed or marinated meat products, processed cheese, salad dressing, bakery products, etc.

Betaine is used in feed to supplement the nutrient intake of fish and shrimps. However, as it is very hygroscopic and highly water-soluble, it is difficult to ensure consistent delivery to fish over extended period of time because the betaine leaches from the feed pellets before they are eaten by the animals. Encapsulation in accordance with the present invention may prevent early dissolution, thus ensuring effective delivery of betaine to fish. Spray cooling cannot, produce microcapsules that retain their content in aqueous media for more than 10-15 minutes, which is not long enough for the fish feed application. These problems are addressed by betaine encapsulated in accordance with the present invention.

8.3 Results

8.3.1 Microscopic analysis

Figure 2 shows a sample of microparticles in accordance with the present invention, as viewed with the light microscope (magnification 200x). The particles are largely spherical, although there are some more irregularly shaped particles, which have resulted from the spray cooling. The inner biphasic core of the particles, (i.e. the w/o solid emulsion) appears as a single mass; it is not possible to observe the matrix nature of the particles from this (and similar) images.

Figure 3 shows a ESEM (Environmental Scanning Electron Microscope) image of similar microparticles in accordance with the present invention, obtained during preliminary investigations. The image on the left shows a network structure, in which two small spheres appear, located in a crater towards the upper left of the image. Towards the right of the image another three small spheres are visible. It is hypothesised that these small spherical particles distributed within the larger, correspond to the gelled aqueous droplets, which contain the active ingredients. The fact that such carrier particles are not completely embedded in the body of the larger particle provides an explanation for the initial release burst of ingredients during the first few minutes.

8.4 Present Invention vs Spray Cooling

As explained herein, the present invention offers advantages over the conventional low-cost but high throughput spray cooling and the more expensive and lower throughput fluid bed process. As shown above the materials of the present invention offer the low production cost, high throughput advantages of spray cooling. It is also demonstrated below that the present invention can approach the slower release rates usually obtained by fluidised bed.

Calcium propionate. The release profile of spray cooled calcium propionate and calcium propionate encapsulated in accordance with the present invention was determined by the basket method by measuring the increase in solution conductivity over 60 minutes. Figure 4 compares the release profiles of two encapsulated and control (unencapsulated) calcium propionate samples (the "release rate" of the control is actually the rate of dissolution of the propionate in the dissolution media). The release rate of spray cooled calcium propionate is consistent with previous experiments: about 75%. is released after 15 minutes. As can be seen, the rate of release of the calcium propionate in accordance with the present invention is significantly slower; only 20% is released after 15 minutes. Even after 1 hour, only 40% of the total amount of calcium propionate available has been release and the rate of release has decreased, suggesting that the a much longer period of time would be required to release the remaining 60% or perhaps even the disruption of the capsule by heat, stress or other triggers.

Citric acid. The release profiles of citric acid samples prepared by spray cooling and in accordance with the present invention were compared by following the pH drop in water as the acid is slowly released from the microcapsules. The concentration of citric acid in the dissolution media as function of time was worked out from the pH, disregarding the polyprotic nature of citric acid. As can be seen from Figure 5 about 80% of spray-cooled citric acid is released after 15 minutes, which is consistent with typical spray cooled samples. It is clearly seen in Figure 5 that the release rate of citric acid from microcapsules in accordance with the present invention is considerably slower than that from the spray-cooled particles. After 40 minutes, only a very small amount of citric acid has been released from the microcapsules in accordance with the present invention. Subsequent boiling of the samples for 20 minutes was required to bring the pH to the final level of the unencapsulated and spray cooled samples. This result demonstrates the increased tightness of the microcapsules in accordance with the present invention,

compared to the spray cooled sample; the fat coating and hydrocolloids have to be completely melted before the bulk of the encapsulated citric acid is released.

Nisin. The release rate of nisin from spray cooled and encapsulated samples in accordance with the invention was measured in an 0,02N HCl aqueous dissolution media at 30°C. Aliquots taken on the first, second and 10th days were analysed by HPLC for nisin content. The analysis quantifies the amount of active nisin present in the dissolution media, which is actually the combination of the nisin released from the microcapsules less the amount degraded over time in the acidic dissolution media. The low pH of the dissolution media minimises, but does not totally prevent degradation of nisin. The release rate of nisin from the spray cooled particles is much slower (days compared to minutes) than typical water-soluble ingredients because the polymeric nature of nisin; diffusion of the nisin through the fat matrix is slowed due to the high molecular weight (3353 g/mol). However, Figure 6 clearly shows that release of nisin from the microcapsules is much slower than from the spray cooled particles: a plateau at 20% release is reached after 2 days for the sample in accordance with the present invention, while the spray cooled sample show a 50% release after 2 days and a further increase to 60% release after 10 days.

8.5 - Effect of hydrocolloids

Betaine was chosen as the model ingredient to study whether the release rate of ingredients encapsulated in accordance with the present invention could be fine-tuned by the choice of the hydrocolloid used to gel the aqueous phase. A series of betaine encapsulated in accordance with the present invention was prepared with various hydrocolloids, which were either gelled upon cooling or cross-linked upon reaction with divalent ions. The release rate was determined by the basket method by taking samples over 60 minutes and analysing by HPLC (using a refractive index detector). Figure 7 shows the release profiles of betaine samples encapsulated in accordance with the present invention, which differ only in the hydrocolloid and thus the gelling mechanism of the inner aqueous phase.

In Figure 7 the data can be seen to fall into three distinct groups. There is a significant difference between the release profiles of the fastest and slowest releasing samples: after 15 minutes, the fastest sample has released twice as much as the slowest sample. Interestingly, the samples that have fast release rates are the samples in which the

aqueous phase is gelled upon cooling (e.g. the hydrocolloids are either carrageenan, pectin 1400, mixtures of LBG and carrageenan or xanthan) while the slow-releasing samples are those in which the aqueous phase has been gelled by crosslinking of the hydrocolloids (alginate or pectin 2580). Agar is an exception; although gelled upon cooling, the release profile ranks closer to the slow-releasing samples.

Table 4. Relaxation times of water in microcapsules of the invention

The effect of the hydrocolloid on the strength of microcapsules in accordance with the present invention was also investigated by pulsed low-field NMR. The relative stability of the gelled or crosslinked aqueous droplets was evaluated by measuring the relaxation time T2 of the water molecules in the inner part of the present microcapsules. The table below shows the time constants fitting best the decay of the NMR intensity after the pulse sequence. The relaxation time is usually directly related to the mobility of the molecule in its environment: a longer relaxation time is associated with a more rigid environment where molecules are restricted in their inter- and intra-molecular translational and rotational movements. The table below shows that the relaxation times of water molecules in gelled carrageenan droplets are shorter than those in cross-linked alginate droplets. This result suggests that the aqueous phase in microcapsules in accordance with the present invention is more rigid when prepared with alginate than with carrageenan. By extension, we can deduce that the other components of the alginate-based aqueous phase are also more restricted in their movement and therefore less likely to diffuse out of the microcapsules, or at least, at a slower rate. This result is consistent with the release profiles of Figure 7, which show the alginate-based microcapsules release their content more slowly than carrageenan-based microcapsules.

Hydrocolloid	T_2 (ms)	
	$T_2(1)$	$T_2(2)$
carrageenan	71,09 ± 0,07	15,8 ± 0,2
alginate	82,5 ± 0,1	20,3 ± 0,2

8.5 - Freeze-triggered release

Microcapsules in accordance with the present invention may typically contain 30-40% water. We have found that water present in the microcapsules may crystallise and expand upon freezing, thus rupturing the fat layer and prompting the release of the

encapsulated ingredient. Thus a freezing-based trigger is provided. Figure 8 shows a transmitted light microscopy image of microcapsules in accordance with the present invention that have been frozen. The image clearly shows that the fat layer has been ruptured upon expansion of the inner water phase during freezing.

8.6 - Application trials

Heat stability of encapsulated nisin in processed cheese

Process	% recovery after 10 min		
	@60°C	@80°C	@100°C
- (unencapsulated)	74	68	59
Spray cooling	75	73	72
Present Invention*	90	84	75

* *Nisin/Alginate sample of Example 8.2*

Nisin in processed cheese Nisin encapsulated in accordance with the present invention was incorporated in a processed cheese formulation and subjected to a heat treatment for 10 minutes. The above table shows that unencapsulated nisin is substantially degraded even at the lowest temperature: 74% and 59% recovery at 60°C and 100°C respectively. Encapsulation in accordance with the present invention and spray cooling are possible routes to reduce the degradation of nisin. As can be seen, spray cooling limited the degradation to around 25% loss at all temperatures, whilst encapsulation in accordance with the present invention provided increased protection at lower temperatures and the similar protection to that conferred by spray cooling at higher temperatures. These trials confirm that encapsulation in accordance with the present invention is a viable means of protection against the thermal degradation of nisin. The present invention is a particularly effective solution at intermediate temperatures.

Pizza Flavours. Frozen pizzas were prepared with 1) no added microencapsulated flavour, 2) a low level (1% w/w on the pizza weight) and 3) a high level (2% w/w on the pizza weight)of pizza flavour encapsulated in accordance with the present invention. The pizzas were then heated 3 minutes at high intensity in a microwave and blind-tested by a "panel". All the members of the panel noticed a much stronger pizza aroma in the room upon opening the microwave door when low or high level of encapsulated flavour had been added to the pizza compared to the controlled pizzas. After the tasting tests, 3

out of 4 members of the panel ranked the flavour intensity of the spiked pizzas slightly higher than the control pizza.

The results suggest that the release of ingredients encapsulated in accordance with the present invention can be triggered by microwave heating. A possible mechanism is that the aqueous droplets in the inner part of the microcapsules absorb the microwave energy, expand and burst out fracture the fat barrier allowing the rapid ingress of water.

Discussion

We have shown that ingredients encapsulated in accordance with the present invention have release profiles significantly and consistently slower than their spray cooled counterparts. Typically, spray cooled samples have release 80% of their content after 15 minutes, while samples in accordance with the present invention have released 20-40% of their contents after the same period of time. Typically it takes only five minutes for spray cooled samples to release half their contents, whereas the present samples reach this limit normally after 60 minutes. The present invnetion is more efficient at delaying the release of the encapsulated ingredient than spray cooling.

We have also shown that a significant portion of the encapsulated ingredient is not released at all in an aqueous environment at room temperature. This contrasts sharply with spray-cooled samples that eventually release all their content within a relatively short time frame, without having to melt the shell material. It is a widely accepted that spray cooled ingredients are released only upon melting of the fat barrier. It has also been established the release of the ingredient can be also triggered by freezing the aqueous phase in the inner part of the microcapsules.

Experiments have also shown that the release profile of an encapsulated sample in accordance with the present invention can be fine tuned by choosing the appropriate hydrocolloid for gelling/crosslinking the aqueous phase.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with

specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in chemistry or related fields are intended to be within the scope of the following claims.